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Performance evaluation of the Roche Elecsys Anti-SARS-CoV-2 S immunoassay

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ABSTRACT

The Elecsys® Anti-SARS-CoV-2 S immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) has been developed for the detection of antibodies to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein. We evaluated the assay performance using samples from seven sites in Germany, Austria, and Switzerland. For specificity and sensitivity analyses, 7880 presumed negative pre-pandemic samples and 827 SARS-CoV-2 PCR-confirmed single or sequential samples from 272 different patients were tested, respectively. The overall specificity and sensitivity (≥14 days post-PCR) for the Elecsys Anti-SARS-CoV-2 S immunoassay were 99.95% (95% confidence interval [CI]: 99.87–99.99; 7876/7880) and 97.92% (95% CI: 95.21–99.32; 235/240), respectively. The Elecsys Anti-SARS-CoV-2 S immunoassay had significantly higher specificity compared with the LIAISON® SARS-CoV-2 S1/S2 IgG (99.95% [2032/2033] vs 98.82% [2009/2033]), ADVIA Centaur® SARS-CoV-2 Total (100% [928/928] vs 86.96% [807/928]), ARCHITECT SARS-CoV-2 IgG (99.97% [2931/2932] vs 99.69% [2923/2932]), iFlash-SARS-CoV-2 IgM (100.00% [928/928] vs 99.57% [924/928]), and EUROIMMUN Anti-SARS-CoV-2 IgG (100.00% [903/903] vs 97.45% [880/903]) and IgA (100.00% [895/895] vs 95.75% [857/895]) assays. The Elecsys Anti-SARS-CoV-2 S immunoassay had significantly higher sensitivity (≥14 days post-PCR) compared with the ARCHITECT SARS-CoV-2 IgG (98.70% [76/77] vs 87.01% [67/77]), iFlash-SARS-CoV-2 IgG (100.00% [76/76] vs 93.42% [71/76]) and IgM (100.00% [76/76] vs 35.53% [27/76]), and EUROIMMUN Anti-SARS-CoV-2 IgG (98.26% [113/115] vs 93.91% [108/115]) assays. Therefore, the Elecsys Anti-SARS-CoV-2 S assay demonstrated a reliable performance across various sample populations for the detection of anti-S antibodies.

1. Introduction

In December 2019 a novel coronavirus emerged (Chan et al., 2020), named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the causative agent of the disease, COVID-19 (Wu et al., 2020; World Health Organization, 2020). SARS-CoV-2 is an enveloped, single-stranded RNA virus of the family Coronaviridae; its genome encodes 16 nonstructural proteins and four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) (Naqvi et al., 2020). The most prominent protein component on the viral surface is the S glycoprotein – a large transmembrane protein that assembles into trimers to form the distinctive surface spikes of coronaviruses (Walls et al., 2020; Tang et al., 2020). Each S monomer consists of two subunits, S1 and S2, which mediate receptor binding (via the receptor-binding domain [RBD] located in S1) and membrane fusion, respectively, leading to entry into host cells (Tang et al., 2020; Wrapp et al., 2020; Ou et al., 2020).

Following SARS-CoV-2 infection, the host mounts an immune response against the virus, including production of specific antibodies

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against viral antigens (Galipeau et al., 2020). Understanding the dynamics of the antibody response to the virus is critical in establishing a relevant time window to use for serology testing (Galipeau et al., 2020). Studies into the kinetics of antibodies to SARS-CoV-2 are rapidly emerging and, both immunoglobulin M (IgM) and G (IgG) antibodies have been detected as early as day 1 after symptom onset (Guo et al., 2020). The chronological order of appearance and levels of IgM and IgG seems to be highly variable and often simultaneous (To et al., 2020; Long et al., 2020; Zhao et al., 2020). Several studies have observed median seroconversion at day 10–13 after symptom onset for IgM and day 12–15 for IgG, with maximum seroconversion for IgM, IgG, and total antibodies occurring at week 2–3, week 2–4, and around week 2, respectively (Long et al., 2020; Zhao et al., 2020; Lou et al., 2020; Young et al., 2020).

Emergence of the COVID-19 pandemic has resulted in an urgent and unmet need to develop reliable serological tests to determine past exposure to the virus and the seroprevalence in a given population (Kontou et al., 2020). This information is crucial to support diagnosis, contact tracing, epidemiological studies, and vaccine development to enable characterization of pre-vaccination immune status and vaccine-induced immune response (Galipeau et al., 2020; Ernst et al., 2021; Zhu et al., 2020; Widge et al., 2021). There are currently 294 candidate SARS-CoV-2 vaccines in development (status August 03, 2021) (World Health Organization, 2021) and, of these, 17 are currently in early, limited, or fully approved use (status July 07, 2021) (Gavi, 2021). The majority of the vaccines in use are based on the S protein, with the goal of eliciting protective neutralizing antibodies; the rest are based on whole inactivated SARS-CoV-2 (Dai and Gao, 2021; Forni and Mantovani, 2021). Serology assays are also needed for the identification of neutralizing antibodies from convalescent plasma donors (Ni et al., 2020).

The Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is an electrochemiluminescence immunoassay (ECLIA), which has been developed for the *in vitro* quantitative detection of antibodies, including IgG, against the SARS-CoV-2 S protein RBD in human serum and plasma (Roche Diagnostics GmbH, 2021a).

The objective of this multicenter European study was to qualitatively evaluate the specificity and sensitivity of the Elecsys Anti-SARS-CoV-2 S immunoassay using pre-pandemic samples (from routine diagnostics or blood donation) and polymerase chain reaction (PCR)-positive samples, respectively, as well as compare the performance of this quantitative test with other commercially available immunoassays in terms of specificity and sensitivity.

2. Materials and methods

2.1. Study design

The study was executed from August 17, 2020 to September 1, 2020 with samples tested at four European sites: Labor Augsburg MVZ GmbH, Augsburg, Germany; MVZ Labor Dr. Limbach & Kollegen GbR, Heidelberg, Germany; Interregionale Blutspende SRK AG (SRK Bern), Bern, Switzerland; and Krankenhaus Barmherzige Brüder, Regensburg, Germany. Samples were collected from those four sites, as well as from three additional sites: Labor Berlin – Charité Vivantes GmbH, Berlin, Germany; Tirol Kliniken, Innsbruck, Austria; and Deutsches Rotes Kreuz Blutspendedienst West, Hagen, Germany.

Samples from Augsburg and Heidelberg included those referred to the respective study site by physicians. Heidelberg also tested samples from employees and hospitalized patients, including a subset from patients receiving dialysis. All samples provided by the study site in Berlin were collected from hospitalized patients, including a subset from patients monitored in the intensive care unit. Samples tested in Regensburg were taken from employees and pediatric patients referred to the site by physicians.

These samples were collected and tested in accordance with applicable regulations, including relevant European Union directives and regulations, and the principles of the Declaration of Helsinki. All samples from Augsburg, Heidelberg, Berlin and Hagen were anonymized. A statement was obtained from the Ethics Committee (EC) of the Landesärztekammer Bayern confirming that there are no objections to the use of anonymized leftover samples. From the EC at the study site in Bern (Switzerland) a waiver was received and from the internal EC at the study site in Innsbruck (Austria) an approval was received. For Regensburg (Germany), EC approvals were already in place, amendments were submitted to notify the EC about Elecsys Anti-SARS-CoV-2 S testing. At Augsburg, Heidelberg, and Bern the assays were performed on the cobas e 801 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland), whereas at Regensburg the assays were performed on the cobas e 601 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). To assess the proper function of cobas e instruments, applications and reagents, a familiarization experiment was performed with quality control material and successfully passed at all measuring sites. Sites were requested to run quality controls after every calibration, for each reagent kit, and at least once every 24 h, and to proceed only if the recovery was within the acceptance criteria.

2.2. Serum and plasma samples

Anonymized frozen, residual serum or plasma samples (≥300 µl volume) from blood donation centers or routine laboratory diagnostics, as well as banked samples, were used for this study. For specificity analysis of the Elecsys Anti-SARS-CoV-2 S assay, 7880 samples (5056 blood donor and 2824 diagnostic routine samples) that were collected before the COVID-19 pandemic in October 2019, and therefore presumed to be negative for SARS-CoV-2, were tested. The diagnostic routine cohort included samples from women attending pregnancy screening and from pediatrics. For the sensitivity analysis of the Elecsys Anti-SARS-CoV-2 S assay, 827 PCR-confirmed single or sequential samples from 272 different patients, with known time difference between blood draw and positive PCR test, were tested. Of these presumed negative and PCR-confirmed samples, 7903 were tested on the commercially available Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) (Roche Diagnostics GmbH, 2021b). Additionally, a number of these samples were tested on other commercially available assays: LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin, Saluggia, Italy) (DiaSorin, 2020), 2052 samples; EUROIMMUN Anti-SARS-CoV-2 IgG (EUROIMMUN, 2020a) and immunoglobulin A (IgA) assays (EUROIMMUN, Lübeck, Germany) (EUROIMMUN, 2020b), 1618 and 1624 samples, respectively; ARCHITECT SARS-CoV-2 IgG (Abbott Laboratories, Abbott Park, Illinois, USA) (Abbott, 2021), 3068 samples; ADVIA Centaur® SARS-CoV-2 Total (Siemens, Tarrytown, New York, USA) (Siemens, 2020), 1064 samples; iFlash-SARS-CoV-2 IgG and IgM assays (YHLO Biotech Co., Ltd, Shenzhen, China) (Shenzhen YHLO Biotech Co Ltd., 2020), both 1062 samples.

2.3. Elecsys Anti-SARS-CoV-2 S assay

The Elecsys Anti-SARS-CoV-2 S immunoassay is a quantitative ECLIA that detects high-affinity antibodies to the SARS-CoV-2 S protein RBD and has a low risk of detecting weakly cross-reactive and unspecific antibodies. Results are automatically reported as the analyte concentration of each sample in U/mL, with <0.80 U/mL interpreted as negative for anti-SARS-CoV-2 S antibodies and ≥0.80 U/mL interpreted as positive for anti-SARS-CoV-2 S antibodies (Roche Diagnostics GmbH. Elecsys Anti-SARS-CoV-2 S assay method sheet. 2020; version 2.0) (Roche Diagnostics GmbH, 2021c).

2.4. Comparator assays

Specimens were analyzed using eight comparator immunoassays according to the manufacturer's instructions. Interpretation of results was performed according to the manufacturer's instructions.

The Elecsys Anti-SARS-CoV-2 assay is an ECLIA for the *in vitro* qualitative detection of antibodies, including IgG, against SARS-CoV-2, using a recombinant protein representing the N antigen (Roche Diagnostics GmbH, 2021b). Results are automatically calculated in the form of a cutoff index (COI), with values <1.0 interpreted as non-reactive (negative) for anti-SARS-CoV-2 N antibodies and ≥1.0 as reactive (positive) for anti-SARS-CoV-2 N antibodies (Roche Diagnostics GmbH, 2021b).

The LIAISON SARS-CoV-2 S1/S2 IgG assay is an indirect chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG anti-S1 and IgG anti-S2 antibodies to SARS-CoV-2 (DiaSorin, 2020). Results are automatically calculated, with antibody concentrations expressed as arbitrary units (AU/mL). Concentrations of <12.0 AU/mL are interpreted as negative, ≥12.0 to <15.0 AU/mL are interpreted as equivocal, and ≥15.0 AU/mL are interpreted as positive (DiaSorin, 2020). Equivocal values are referred to as 'gray zone' results.

The EUROIMMUN Anti-SARS-CoV-2 IgG and IgA assays are separate enzyme-linked immunosorbent assays (ELISAs) that detect IgG or IgA anti-S1 antibodies to SARS-CoV-2 (EUROIMMUN, 2020a, b). Results are evaluated semi-quantitatively by calculation of a ratio in which the absorbance values of the controls or patient samples are related to the absorbance value of the calibrator (EUROIMMUN, 2020a, b). For both assays, ratio results <0.8 are interpreted as negative, ≥0.8 to <1.1 are borderline, and ≥1.1 are positive (EUROIMMUN, 2020a, b). Borderline values are referred to as 'gray zone' results.

The ARCHITECT SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG antibodies against the N antigen (Abbott, 2021). Results are expressed in signal-to-cutoff (S/CO) values, with <1.4 results interpreted as negative and ≥1.4 results interpreted as positive (Abbott, 2021).

The ADVIA Centaur SARS-CoV-2 Total assay is a CLIA intended for the qualitative detection of antibodies against the RBD of the S1 protein (Siemens, 2020). Results are reported in index values, with <1.0 interpreted as non-reactive (negative) for anti-SARS-CoV-2 antibodies and ≥1.0 interpreted as reactive (positive) for anti-SARS-CoV-2 antibodies (Siemens, 2020).

The iFlash-SARS-CoV-2 IgM and IgG assays (Shenzhen YHLO Biotech Co Ltd., 2020) are separate CLIAs used for the qualitative detection of IgM or IgG against the S and N proteins. The iFlash system automatically calculates the analytic concentration of each sample, with <10 AU/mL interpreted as non-reactive and ≥10 AU/mL interpreted as reactive for anti-SARS-CoV-2 IgM or IgG antibodies.

2.5. Statistical analysis

Sample size estimations for specificity and sensitivity analyses were based on formulae proposed previously (Hajian-Tilaki, 2014). Assuming specificities between 0.998 and 0.999 and a sensitivity of 0.999, samples sizes of 1698–20964 and 32–50 respectively, would be required to obtain a significance level of 0.05 and a power of 0.8. For specificity and sensitivity calculations, point estimates and two-sided 95% confidence intervals (CIs) using the exact method were computed employing R version 3.4.0 (R Core Team, 2017). In the sensitivity evaluation, assay results were assigned to the respective week after positive PCR result. In the comparison with other commercially available assays, only samples with paired measurements were included in the respective analyses. For the differences in estimated specificities and sensitivities between Elecsys Anti-SARS-CoV-2 S assay and the comparator assays, two-sided 95% Wald CIs were calculated as previously recommended (Wenzel and Zapf, 2013). If these CIs did not include zero, differences were considered as statistically significant.

3. Results

3.1. Overall performance of the Elecsys Anti-SARS-CoV-2 S assay

3.1.1. Specificity in different target cohorts

Specificity of the Elecsys Anti-SARS-CoV-2 S assay was evaluated at three European sites (with samples from five European sites) using 7880 evaluable residual samples from blood donors and routine diagnostic testing; all of which were collected before October 2019 and presumed negative for SARS-CoV-2 antibodies. The overall specificity for all samples was 99.95% (95% CI: 99.87–99.99 [7876/7880]) (Table 1). There were four samples with reactive results of 1.790 U/mL, 0.900 U/mL, 0.870 U/mL, and 1.130 U/mL. Three of these reactive samples were from blood donor samples, of which one was collected in March 2016 (influenza season) at Innsbruck, Austria and two were collected in July/August 2018 (outside influenza season) at Bern, Switzerland (Table 1). There was no statistically significant difference in specificity between blood donor samples collected during or outside influenza season. The other reactive sample was from the pregnancy screening cohort in Augsburg (Table 1).

3.1.2. Sensitivity in different target cohorts

In total, 827 single and sequential samples from 272 SARS-CoV-2 PCR-confirmed patients were evaluated at three European sites (with samples from four European sites). The time span of samples collected after positive PCR was between day 0 and day 120. For subjects with sequential blood draws with more than one sample per time interval, only the result of the last blood draw per given time interval was used for the respective sensitivity calculation. The sensitivity of the Elecsys Anti-SARS-CoV-2 S assay ≥14 days post-PCR (n = 240) was 97.92% (95% CI:

Table 1
Specificity results for the Elecsys Anti-SARS-CoV-2 S assay.

Sample cohort		Reactive samples/ number of samples	Specificity (95% confidence intervals, 2-sided)
Blood donors			
Origin / season	Innsbruck / influenza season	1/1050	99.90% (99.47–100.00)
	Hagen / no seasonal selection	0/955	100.00% (99.61–100.00)
	Bern / outside influenza season	2/2000	99.90% (99.64–99.99)
	Bern / influenza season	0/1051	100.00% (99.65–100.00)
Total blood donors		**3/5056**	**99.94% (99.83–99.99)**
Diagnostic routine			
Origin / cohort	Augsburg / diagnostic routine	0/400	100.00% (99.08–100.00)
	Augsburg / pregnancy screening	1/1496	99.93% (99.63–100.00)
	Heidelberg / pregnancy screening	0/737	100.00% (99.50–100.00)
	Heidelberg / pediatric samples	0/191	100.00% (98.09–100.00)
Total diagnostic routine		**1/2824**	**99.96% (99.80–100.00)**
Overall (all samples)		**4/7880**	**99.95% (99.87–99.99)**

Table 2
Overall sensitivity results for the Elecsys Anti-SARS-CoV-2 S assay.

Days post-PCR-positive test	Non-reactive samples/ number of samples	Sensitivity (95% confidence intervals, 2-sided)
0 to 6	19/44	56.82% (41.03–71.65)
7 to 13	7/49	85.71% (72.76–94.06)
14 to 20	1/47	97.87% (88.71–99.95)
21 to 27	0/58	100.00% (93.84–100.00)
28 to 34	0/43	100.00% (91.78–100.00)
35 to 41	1/57	98.25% (90.61–99.96)
42 to 48	0/47	100.00% (92.45–100.00)
49 to 55	4/39	89.74% (75.78–97.13)
56 to 62	0/33	100.00% (89.42–100.00)
≥63 (up to 120)	0/21	100.00% (83.89–100.00)
All samples ≥14 (up to 120)[a]	**5/240**	**97.92% (95.21–99.32)**

PCR, polymerase chain reaction.
[a] Only the last sample taken ≥14 days post-PCR of each patient is included in the sensitivity calculation.

95.21–99.32 [235/240]) (Table 2). The resulting site-specific sensitivities for Augsburg, Berlin, Heidelberg, and Regensburg samples collected ≥14 days post-PCR confirmation were 100.00% (95% CI: 95.89–100.00), 100.00% (95% CI: 91.40–100.00), 98.72% (95% CI: 93.06–99.97%), and 87.88% (95% CI: 71.80–96.60), respectively.

3.1.3. Determination of seroconversions

For all subjects with at least two sequential blood draws, trajectories were plotted to determine antibody titer development from day 0–78 post-PCR-positive test (Fig. 1). Most trajectories showed a rapid increase in antibody titer and no considerable decline of antibody titer was seen for the early and later blood draws. Once detected reactive, none of the subsequent samples drawn per subject showed a decline of titer below the cutoff.

3.2. Comparison with Elecsys Anti-SARS-CoV-2 assay

A direct method comparison between the Elecsys Anti-SARS-CoV-2 S assay and the commercially available Elecsys Anti-SARS-CoV-2 assay was performed. This included a total of 7903 samples comprising both confirmed positive samples from sensitivity testing and presumed positive samples with at least one positive antibody result (n = 1011), as well as presumed negative samples from specificity testing cohort samples (n = 6892: n = 4068 blood donors; n = 2824 routine diagnostic). For all samples, the overall percent agreement (OPA) between the Elecsys Anti-SARS-CoV-2 S assay and the Elecsys Anti-SARS-CoV-2 assay was 99.30% (95% CI: 99.10–99.48) (Supplemental Table 1).

3.3. Comparison with other commercially available assays

The performance of the Elecsys Anti-SARS-CoV-2 S immunoassay was compared with seven other commercially available SARS-CoV-2 assays, and sensitivity and specificity results, along with percent agreement, were recorded. Information regarding the different assays, including the technique, target, and Ig measured can be found in Supplemental Table 2.

The OPA between the Elecsys Anti-SARS-CoV-2 S assay and other comparator tests was recorded (Supplemental Table 2). The Elecsys Anti-SARS-CoV-2 S test had the highest OPA with the ARCHITECT SARS-CoV-2 IgG (N-assay), at 99.19% (95% CI: 98.80–99.47), and the lowest OPA with the ADVIA Centaur SARS-CoV-2 Total (S-assay), at 88.25% (95% CI: 86.16–90.13) (Supplemental Table 3).

3.3.1. Specificity

The specificity of the Elecsys Anti-SARS-CoV-2 S assay was comparable or higher than the specificity of all tested comparator assays (Table 3). The specificity of the Elecsys Anti-SARS-CoV-2 S test was significantly higher compared with the LIAISON SARS-CoV-2 S1/S2 IgG (99.95% [2032/2033] vs 98.82% [2009/2033]), ADVIA Centaur SARS-CoV-2 Total (100% [928/928] vs 86.96% [807/928]), ARCHITECT SARS-CoV-2 IgG (99.97% [2931/2932] vs 99.69% [2923/2932]), iFlash-SARS-CoV-2 IgM (100.00% [928/928] vs 99.57% [924/928]), and EUROIMMUN Anti-SARS-CoV-2 IgG (100.00% [903/903] vs 97.45% [880/903]) and IgA (100.00% [895/895] vs 95.75% [857/895]) assays (Table 3, Supplemental Table 4A). No statistically significant difference was observed between the specificity of the Elecsys Anti-

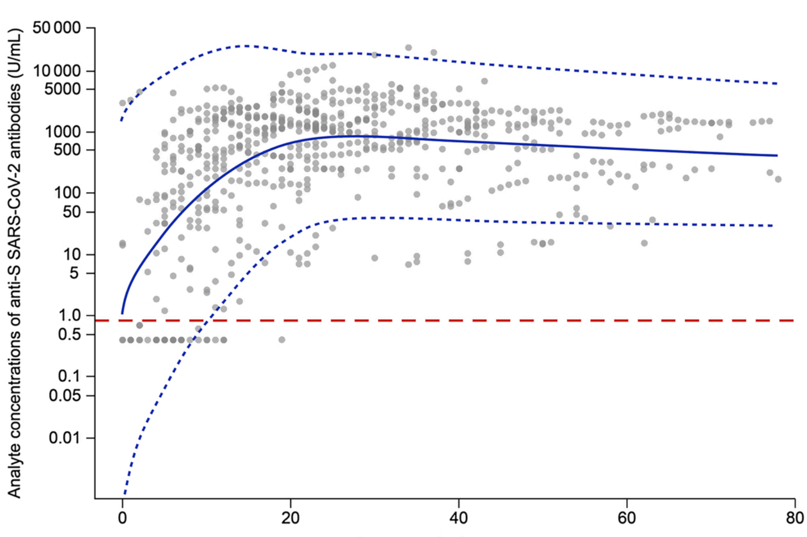

Fig. 1. Longitudinal antibody titers of all subjects. Concentration of anti-S SARS-CoV-2 antibodies, as measured by the Elecsys Anti-S SARS-CoV-2 S immunoassay, over time (days 0 to 78) in sequential samples from all study sites. Each grey circle represents a different data point, with darker circles representing overlapping data points. The solid blue line represents the combined curve, and the blue dashed lines represent the upper and lower confidence limits. The red dashed line indicates the assay cutoff limit (0.80 U/mL).

Table 3
Specificity of the Elecsys Anti-SARS-CoV-2 S assay and other comparator assays.

Assay	Reactive samples/ number of samples	Specificity (95% confidence intervals, 2-sided)
Comparison with anti-S assays		
LIAISON SARS-CoV-2 S1/S2 IgG (GZ excl.)[a]	24/2033	98.82% (98.25–99.24)
Elecsys Anti-SARS-CoV-2 S	1/2033	99.95% (99.73–100.00)
LIAISON SARS-CoV-2 S1/S2 IgG (GZ+)[a]	31/2040	98.48% (97.85–98.97)
Elecsys Anti-SARS-CoV-2 S	1/2040	99.95% (99.73–100.00)
ADVIA Centaur SARS-CoV-2 Total	121/928	86.96% (84.62–89.06)
Elecsys Anti-SARS-CoV-2 S	0/928	100.00% (99.60–100.00)
EUROIMMUN Anti-SARS-CoV-2 IgG (GZ excl.)	23/903	97.45% (96.20–98.38)
Elecsys Anti-SARS-CoV-2 S	0/903	100.00% (99.59–100.00)
EUROIMMUN Anti-SARS-CoV-2 IgG (GZ+)	44/924	95.24% (93.66–96.52)
Elecsys Anti-SARS-CoV-2 S	0/924	100.00% (99.60–100.00)
EUROIMMUN Anti-SARS-CoV-2 IgA (GZ excl.)	38/895	95.75% (94.22–96.98)
Elecsys Anti-SARS-CoV-2 S	0/895	100.00% (99.59–100.00)
EUROIMMUN Anti-SARS-CoV-2 IgA (GZ+)	71/928	92.35% (90.45–93.98)
Elecsys Anti-SARS-CoV-2 S	0/928	100.00% (99.60–100.00)
Comparison with anti-N assay		
ARCHITECT SARS-CoV-2 IgG	9/2932	99.69% (99.42–99.86)
Elecsys Anti-SARS-CoV-2 S	1/2932	99.97% (99.81–100.00)
Comparison with anti-S and -N assays		
iFlash-SARS-CoV-2 IgG	0/928	100.00% (99.60–100.00)
Elecsys Anti-SARS-CoV-2 S	0/928	100.00% (99.60–100.00)
iFlash-SARS-CoV-2 IgM	4/928	99.57% (98.90–99.88)
Elecsys Anti-SARS-CoV-2 S	0/928	100.00% (99.60–100.00)

GZ, gray zone.
[a] For antibody assays with a gray zone, two calculations were performed. In the first calculation, all gray zone results were excluded from the analysis (GZ excl.) and in the second calculation these results were interpreted as reactive (GZ+).

SARS-CoV-2 S assay compared with the iFlash-SARS-CoV-2 IgG assay, which was 100.00% (928/928) for both assays (Table 3, Supplemental Table 4A).

3.3.2. Sensitivity

The sensitivity of the Elecsys Anti-SARS-CoV-2 S assay for detecting seropositive results was compared with six comparator assays; analysis compared with the LIAISON SARS-CoV-2 S1/S2 IgG test could not be performed due to a small sample size. Sensitivity was recorded for samples collected between 0–6, 7–13, and ≥14 days post-PCR-positive test (Table 4). The EUROIMMUN Anti-SARS-CoV-2 IgA assay showed a higher sensitivity in the 0–6 (59.09% [26/44] vs 56.82% [25/44]) and 7–13 days (91.84% [45/49] vs 85.71% [42/49]) post-PCR time intervals and a lower sensitivity in the ≥14 days (97.46% [115/118] vs 99.15% [117/118]) post-PCR time interval compared with the Elecsys Anti-SARS-CoV-2 S assay (Table 4). The sensitivity of the Elecsys Anti-SARS-CoV-2 S assay at detecting antibodies ≥14 days post-PCR was significantly higher compared with the ARCHITECT SARS-CoV-2 IgG (98.70% [76/77] vs 87.01% [67/77]), iFlash-SARS-CoV-2 IgG (100.00% [76/76] vs 93.42% [71/76]) and IgM (100.00% [76/76] vs 35.53% [27/76]), and EUROIMMUN Anti-SARS-CoV-2 IgG (98.26% [113/115] vs 93.91% [108/115]) assays (Table 4, Supplemental Table 4B).

4. Discussion

Due to the COVID-19 pandemic, there is a pressing need to develop highly specific and sensitive serology tests to assist with the diagnosis of, and to reveal past exposure to, the SARS-CoV-2 virus (Kontou et al., 2020), as well as to support the development of vaccines through distinguishing natural infection-induced immunity from vaccine-induced immunity (Galipeau et al., 2020; Ernst et al., 2021). This was the first multicenter study to demonstrate the performance of the automated Elecsys Anti-SARS-CoV-2 S immunoassay, which detects antibodies against the SARS-CoV-2 S protein RBD. Antibodies against the RBD have previously been shown to correlate strongly with protective neutralizing antibodies (Premkumar et al., 2020).

The results from our study revealed that the Elecsys Anti-SARS-CoV-2 S immunoassay displays a robust performance under routine conditions at multiple sites in Europe, with a very high specificity (99.95% [7876/7880]) and sensitivity (97.92% [235/240]) for the detection of anti-S antibodies. The point estimates for specificity and sensitivity are comparable to the values reported in the package insert of the Elecsys Anti-SARS-CoV-2 S assay (99.98% and 98.8%, respectively) (Roche Diagnostics GmbH, 2021a). In addition, the Elecsys Anti-SARS-CoV-2 S assay showed a performance comparable with the commercially available Elecsys Anti-SARS-CoV-2 (N-assay), with 95% CIs that overlap (99.69–99.88% for specificity and 97.0–100% for sensitivity); both assays had a very high overall percent agreement. The Elecsys Anti-SARS-CoV-2 assay has a previously reported specificity and sensitivity ≥14 days post-confirmation of 99.8% and 99.5%, respectively (Muench et al., 2020). These data indicate that the Elecsys Anti-SARS-CoV-2 S assay can be utilized to meet the current need for anti-SARS-CoV-2 antibody testing, alongside or in place of the Elecsys Anti-SARS-CoV-2 (N) assay, depending on the study objectives.

The overall specificity of >99.9% determined in this study demonstrated that the Elecsys Anti-SARS-CoV-2 S is a highly specific assay for the detection of antibodies against SARS-CoV-2. Notably, this analysis included 2424 samples from pregnant women and pediatric populations. The availability of an accurate SARS-CoV-2 serology assay is particularly important for the pregnant population, considering the changes in the immune system that occur during pregnancy, which may increase the woman's susceptibility to severe infection (Dashraath et al., 2020). Additionally, an antibody assay with a high specificity is imperative to reduce the risk of false-positive results, which may inaccurately indicate a past SARS-CoV-2 infection (Farnsworth and Anderson, 2020). Our

Table 4
Sensitivity of the Elecsys Anti-SARS-CoV-2 S assay and other comparator assays.

Assay	Days post-PCR-positive test	Non-reactive samples/number of samples	Sensitivity (95% confidence intervals, 2-sided)
Comparison with anti-S assays			
ADVIA Centaur SARS-CoV-2 Total	0–6	8/18	55.56% (30.76–78.47)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	4/77	94.81% (87.23–98.57)
Elecsys Anti-SARS-CoV-2 S	0–6	8/18	55.56% (30.76–78.47)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	1/77	98.70% (92.98–99.97)
EUROIMMUN Anti-SARS-CoV-2 IgG (GZ excl.)[a]	0–6	21/43	51.16% (35.46–66.69)
	7–13	7/45	84.44% (70.54–93.51)
	≥14	7/115	93.91% (87.86–97.52)
Elecsys Anti-SARS-CoV-2 S	0–6	18/43	58.14% (42.13–72.99)
	7–13	7/45	84.44% (70.54–93.51)
	≥14	2/115	98.26% (93.86–99.79)
EUROIMMUN Anti-SARS-CoV-2 IgG (GZ+)[a]	0–6	21/44	52.27% (36.69–67.54)
	7–13	7/49	85.71% (72.76–94.06)
	≥14	6/119	94.96% (89.35–98.13)
Elecsys Anti-SARS-CoV-2 S	0–6	19/44	56.82% (41.03–71.65)
	7–13	7/49	85.71% (72.76–94.06)
	≥14	1/119	99.16% (95.41–99.98)
EUROIMMUN Anti-SARS-CoV-2 IgA (GZ excl.)	0–6	18/44	59.09% (43.25–73.66)
	7–13	4/49	91.84% (80.40–97.73)
	≥14	3/118	97.46% (92.75–99.47)
Elecsys Anti-SARS-CoV-2 S	0–6	19/44	56.82% (41.03–71.65)
	7–13	7/49	85.71% (72.76–94.06)
	≥14	1/118	99.15% (95.37–99.98)
EUROIMMUN Anti-SARS-CoV-2 IgA (GZ+)	0–6	18/44	59.09% (43.25–73.66)
	7–13	4/49	91.84% (80.40–97.73)
	≥14	3/121	97.52% (92.93–99.49)
Elecsys Anti-SARS-CoV-2 S	0–6	19/44	56.82% (41.03–71.65)
	7–13	7/49	85.71% (72.76–94.06)
	≥14	1/121	99.17% (95.48–99.98)
Comparison with anti-N assay			
ARCHITECT SARS-CoV-2 IgG	0–6	9/18	50.00% (26.02–73.98)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	10/77	87.01% (77.41–93.59)
Elecsys Anti-SARS-CoV-2 S	0–6	8/18	55.56% (30.76–78.47)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	1/77	98.70% (92.98–99.97)
Comparison with anti-S and -N assays			
iFlash-SARS-CoV-2 IgG	0–6	8/17	52.94% (27.81–77.02)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	5/76	93.42% (85.31–97.83)
Elecsys Anti-SARS-CoV-2 S	0–6	7/17	58.82% (32.92–81.56)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	0/76	100.00% (95.26–100.00)
iFlash-SARS-CoV-2 IgM	0–6	11/17	35.29% (14.21–61.67)
	7–13	9/11	18.18% (2.28–51.78)
	≥14	49/76	35.53% (24.88–47.34)
Elecsys Anti-SARS-CoV-2 S	0–6	7/17	58.82% (32.92–81.56)
	7–13	2/11	81.82% (48.22–97.72)
	≥14	0/76	100.00% (95.26–100.00)

GZ, gray zone.
[a] For antibody assays with a gray zone, two calculations were performed. In the first calculation, all gray zone results were excluded from the analysis (GZ excl.) and in the second calculation these results were interpreted as reactive (GZ+).

study did not assess the specificity of the Elecsys Anti-SARS-CoV-2 S assay using samples from individuals with other respiratory infections. However, this assay has been previously tested using 1468 samples containing potentially cross-reactive samples collected before October 2019, and 100% specificity was obtained with no cross-reactivity (Roche Diagnostics GmbH, 2021c). These samples were from individuals confirmed to be infected with MERS-CoV, common cold coronaviruses, or other respiratory infections (Roche Diagnostics GmbH, 2021c).

The Elecsys Anti-SARS-CoV-2 S immunoassay demonstrated good specificity (99.95–100.00%) and sensitivity at ≥14 days post-PCR-positive test (98.26–100.00%) in direct comparison with other commercially available assays; both performance measurements were equal to or greater than those for other evaluated comparator assays. These other assays have also been assessed in previous studies (Kontou et al., 2020; Meyer et al., 2020; Kohmer et al., 2020; Okba et al., 2020; Ocmant et al., 2021). However, it is important to note that, for a direct comparison of sensitivity, the available assays differ with respect to assay designs (e.g. antibody classes used) as well as the targets (anti-N and anti-S) that they detect.

A multicenter comparison of seven serology assays, including the Elecsys Anti-SARS-CoV-2 assay, revealed a subpopulation of PCR-confirmed SARS-CoV-2 individuals who were persistently seronegative, which represents a proportion of patients who may be at risk for re-infection (Oved et al., 2020). Within the group of PCR-confirmed samples in our study, for which there was at least one S and one N antibody result from another comparator assay, there were samples from five patients all of which had a reactive Elecsys Anti-SARS-CoV-2 S ECLIA result, a reactive or 'gray zone' EUROIMMUN ELISA result and a reactive ADVIA Centaur CLIA result (S-assays), and a non-reactive Elecsys Anti-SARS-CoV-2 ECLIA and ARCHITECT SARS-CoV-2 IgG CMIA result (N-assays). There was only one sample that was commonly non-reactive in all S-based assays and reactive in all N-based assays. However, the blood draw was taken very early, on the same day as the PCR was done, and the follow-up sample was reactive with the Elecsys Anti-SARS-CoV-2 S assay. Data indicating differences in the kinetics of serology assays are ambiguous and there was little difference in the timing of these responses (Kohmer et al., 2020; Wang et al., 2020; Van Elslande et al., 2020; Johnson et al., 2020; Burbelo et al., 2020). However, differences between N- and S- antigen-based assays should be taken into consideration when interpreting results.

A major strength of this study is the large cohort of presumed negative samples collected before the COVID-19 pandemic from multiple sites that were used to determine the specificity of the assay, as well as the multiple method comparison analyses performed and the various population cohorts used, with samples from pediatrics and from pregnant women. Further studies should be performed to determine the sensitivity of the Elecsys Anti-SARS-CoV-2 S immunoassay on a larger sample group.

The Elecsys Anti-SARS-CoV-2 S assay could be used to support studies requiring quantification of antibody responses and vaccine efficacy studies and may be particularly useful due to the majority of vaccines being based on the S protein. Previous studies have utilized the Elecsys Anti-SARS-CoV-2 S immunoassay to determine the immune response to vaccination in various populations (Salvagno et al., 2021; Kennedy et al., 2021; Herishanu et al., 2021; Cavalcanti et al., 2021; Callegaro et al., 2021; Seyahi et al., 2021), as well as to quantify antibody levels (Rubio-Acero et al., 2021; Resman Rus et al., 2021), although further evaluation is necessary to assess the performance of the Elecsys Anti-SARS-CoV-2 S immunoassay for these specific purposes as assessing vaccine efficacy is currently outside of the intended use of the assay.

5. Conclusion

This study demonstrated the performance of the Elecsys Anti-SARS-CoV-2 S immunoassay, with a very high specificity of 99.95% (7876/7880) and sensitivity of 97.92% (235/240) in samples ≥14 days post-PCR confirmation, which was comparable to other commercially available immunoassays. Therefore, these data support the use of the Elecsys Anti-SARS-CoV-2 S immunoassay for reliable identification of past exposure to SARS-CoV-2 in various populations, and highlight the potential for the use of this assay in determining immune status during vaccine efficacy studies.

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Data availability

Qualified researchers may request access to individual patient level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here: https://vivli.org/members/ourmembers/. For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

Ethics approval

The samples used in this study were collected and tested in accordance with applicable regulations, including relevant European Union directives and regulations, and the principles of the Declaration of Helsinki. All samples from Augsburg, Heidelberg, Berlin and Hagen were anonymized. A statement was obtained from the Ethics Committee (EC) of the Landesärztekammer Bayern confirming that there are no objections to the use of anonymized leftover samples. From the EC at the study site in Bern (Switzerland) a waiver was received and from the internal EC at the study site in Innsbruck (Austria) an approval was received. For Regensburg (Germany), EC approvals were already in place, amendments were submitted to notify the EC about Elecsys Anti-SARS-CoV-2 S testing.

Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2021.114271.

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